Betalactam resistance in Gram-negative bacteria

P1214 Extended Spectrum β-lactamases (ESBs) from Gram Negative Bacilli Isolated from Srinagarind Hospital, Thailand

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Objectives: To study the prevalence of ESBs in certain genera of gram negative bacteria which are commonly isolated from patients of Srinagarind Hospital in northeast of Thailand, and determine the minimum inhibitory concentration (MIC) of cephalothin (CP), cefotaxime (CTX), cetazidime (CAZ), ceftriaxone (CRO) and cefoperazone (CFP).

Methods: Two hundreds strain of bacteria in family *Enterobacteriaceae* and one hundred strain of bacteria in family *Pseudomonadaceae* isolated from clinical specimens were studied. The test for ESBs was done by double disk diffusion procedure. The MIC was studied by standard agar dilution method with *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as the control.

Results: The results showed that 52 from 200 strains of *Enterobacteriaceae* and 28 from 100 strains of *Pseudomonadaceae* produced ESBs (26% and 28% respectively). 63 of 80 ESBs producing strains have the MIC of CP, CTX, CAZ, CRO and CFP above the cut off point (>8 ug/ml) and 17 strains have the MIC lower than the cut off point. The ESBs non producing strains have the MIC distributed from 0.5 ug/ml to 64 ug/ml with the majority of strains were susceptible.

Conclusions: About 26 to 28 percents of common gram negative bacteria from clinical specimens were ESBs positive. About 78 percents of the ESBs producing strains resist to the antibiotics studied.

P1215 Detection of the Extended-Spectrum Beta-Lactamase (ESBL) Producing *Klebsiella Pneumoniae* Clinical Isolates

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Objectives: Extended spectrum beta-lactamases are sometimes difficult to detect by routine tests. We used E test strips to detect them and isoelectrofocusing analysis to identify their production.

Methods: We selected 20 unrelated suspected ESBL-producing isolates of *Klebsiella pneumoniae* that were susceptible (S) or intermediate (I) to cefoxitin and resistant (R) to one or more of the following: ceftazidime (Caz), cefotaxime (Ctx) and aztreonam (Atm) and tested by E test ESBL strip. This strip (AB Biodisk, Solna, Sweden) has two ceftazidime gradients on opposite ends of the strip, with clavulanic acid added to one of the gradients. An MIC ratio of Caz/Caz + clavulanic acid (CazCl) ≥16 is claimed to indicate the presence of an ESBL. The method was also tested with known ESBL strain.

Results: The E test ESBL strip results showed that 19/20 of the isolates to be ESBL-producers with MIC ratios of ≥16 for Caz/CazCl. B-lactamases with pls 8.2 and 7.6 were found by isoelectric focusing in the tested isolates.

Conclusions: E test ESBL strip appears to be a generally reliable, less subjective in the interpretation than the double disk diffusion test and technically simple method for the identification and/or confirmation of ESBLs.

P1216 Antibiotic Resistance and β-Lactamase production by *Salmonella* and *Shigella* Isolated in Tripoli-Libya

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Objectives: To provide reliable information about the antibiotic susceptibility of *Salmonella* and *Shigella* species isolated in Tripoli, Libya and about their ability to produce β-lactamase.

Methods: Included in this study 22 *Salmonella* and 11 *Shigella* isolated from the stool of children with or without diarrhoea aged less than 3 years. β-lactamase was detected by the iodometric method and antibiotic sensitivity by the disc diffusion and method.

Results: Of the 22 *Salmonella* and 12 *Shigella* tested, 8 (36%) and 4 (33%) produced β-lactamase, 11 (50%) and 4 (36%) were resistant to ampicillin, 11 (50%) and 3 (27%) to chloramphenicol, 9 (41%) and 0 (0.0%) to gentamicin, 10 (45%) and 1 (8%) to kanamycin, 4 (18%) and 10 (91%) to tetracycline and 10 (45%) and 7 (64%) to trimethoprim-sulphamethoxazole. All isolates were susceptible to ceftriaxone, ciprofloxacin, nalidixic acid and polymyxin B.

Conclusions: Due to the high rates of resistance to the commonly used antibiotics and production of β-lactamases by *Salmonella* and *Shigella* isolated in Tripoli, other drugs as ciprofoxacin should be considered as drugs of choice for the treatment of infections caused by these organisms.

P1217 Cloning and Nucleotide Sequence Analysis of a Gene Coding an OXA Type β-lactamase

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Objective: To clone and analyse the nucleotide sequence of a gene from *A. baumannii* coding an OXA type β-lactamase.

Methods: Isoelectricfocusing was performed on a clinical strain of *A. baumannii* which showed resistance to all β-lactam antibiotics. PCR was carried out with integron specific primers and the amplification product cloned into a pCRII vector. The clone was progressively sequenced. Susceptibility testing was performed by the agar dilution method in accordance with the NCCLS guidelines.

Results: A novel derived β-lactamase (OXA type) was found in an integron in *A. baumannii* which also contained an *aad*B gene. The isoelectric point was 7.0 and its nucleotide sequence differed from that of OXA 3 in 4 bp. However, 2 of these 4 changes were silent. The other two mutations generated a substitution of Phe 59 to Leu and Ile 217 to Met. The latter change has also been observed between OXA 2 and its derivative OXA 15. The amino acid sequence homology between this OXA and OXA 3 was of 99.27%. This enzyme confers resistance to cephalotin, ticarcillin, piperacillin and ampicillin. Biochemical characterization of the β-lactamase is currently in progress.

Conclusions: A new OXA type β-lactamase has been found. It is the first of its class to be described in *A. baumannii* and the first OXA 3 derivative found. Therefore, this new enzyme could be called OXA 19.

P1218 Detection of Extended Spectrum β-lactamases in Clinical Isolates of *Enterobacter* spp. in Italy

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Objectives: *E. cloacae* and *E. aerogenes* are frequently isolated from

patients in ICUs, clinical and surgical wards in our geographical area and the recovery of strains resistant to extended spectrum β-lactams is increasingly frequent. Derepression or stable derepression of AmpC β-lactamase represents the widely reported mechanism responsible for resistance to newer generation cephalosporins. ESβLs are rarely detected in *Enterobacter* spp. We assessed whether these enzymes represent a significant cause of resistance in this genus.

Methods: *Enterobacter* spp. strains were collected from two different hospitals in Northern Italy over two years. There is currently no reliable method designed for detection of ESβLs in isolates of *Enterobacteriaceae* other than *E. coli* and *Klebsiella* spp.; since the cefepime retains reasonable activity against derepressed enterobacteria but is labile to hydrolysis by ESβLs we suspected the ESβL production when the isolates showed resistance or reduced susceptibility to cefepime. The double disk test was performed using amoxicillin-clavulanate and ceftazidime, cefotaxime, cefepime, aztreonam disks placed 30–22 mm centre to centre. Production of chromosomal and plasmid mediated β-lactamases was also studied in crude cellular extracts. The enzymes extracted from clinical isolates and related transconjugants were characterized by biochemical and molecular methods.

Results: All the strains showing an enhancement of the zone of inhibition of cefepime by clavulanate resulted to be producing TEM or SHV derivative β-lactamases plasmid mediated in addition to AmpC β-lactamase.

Conclusions: In our geographical area the interplay of AmpC and TEM or SHV derivative β-lactamases may account for enhanced resistance to β-lactams.

P1219 Betalactamase of *Prevotella* Species: Characterization and Substrate Profile

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Prevotella are increasingly encountered in clinical specimen. We report a study of 306 strains isolated from clinical specimens in our laboratory. 157 strains were betalactamase secretor. The MICs of Amoxicillin, Amoxicillin more clavulanic acid, Piperacillin, Cefuroxim, Cefotaxim, Cefotaxim more sulbactam and Cefoxitin were determined by agar dilution method according to NCCLS recommendation.

Nine betalactamase positive strains were examined for enzymatic substrat profile. The cultures were performed on agar. The cells were harvested after 40 to 60 hr of incubation. The enzyme, were extracted by sonication. The cephalosorinase activity, of the crude extracts was measured by spectrophotometric method with nitrocephin and the ratio Vm/Km was determined. The betalactamase positive strains have relatively high MICs of Ampicillin, Piperacilline Cefuroxim and Cefotaxim. All enzymes hydrolysed Cephalotin, Cefuroxim and Cefotaxim, but weakly and slowly Ampicillin. None of there enzymes hydrolysed Cefoxitin. Addition of clavulanate or sulbactam led to a significant decrease of the MICs.

Specific activity of extract appeared correlated with MICs to Ampicilline and Cefuroxim.

P1220 Hydrolysis of Cephalosporins and Carbapenems by *Sphingobacterium multivorum* Strains

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Objectives: *Sphingobacterium (Flavobacterium) multivorum* is a rare causal agent of nosocomial infections in hospitalised patients.

Methods: Three *S. multivorum* strains isolated in Dérer's Hospital in Bratislava were identified using the SCEPTOR (Becton Dickinson). Antimicrobial susceptibility testing was performed by disc diffusion test. The relative rate of hydrolysis (Vmax) of antibiotics was estimated by the macro-iodometric method. ESBLs profile of strains was performed using double disc diffusion test.

Results: *S. multivorum* No. 283 and 137 (resistant to cephalotine, cefotaxime, ceftazidime, imipenem, meropenem and aztreonam), unlike the No. 280 (resistant to cephalotine, imipenem amd meropenem), produced ESBLs. Moreover, mutant colonies of the strain No. 137, resistant to clavulanate inhibition of the ESBL, appeared in the zones of inhibited growth around discs of cefotaxime and ceftazidime. The hydrolysis of cefotaxime and ceftazidime was inhibited by clavulanate in the sonicates of strains No. 283 and 137. Hydrolysis of imipenem was inhibited by EDTA in both strains. The mutant colonies of the strain No. 137 were inhibited by EDTA, but not by clavulanate.

Conclusions: From results it might be concluded, that *S. multivorum* strains No. 283 and 137 produce metallo-β-lactamase conferring them resistance to carbapenems and, ESBLs hydrolysing cefotaxime and ceftazidime inhibited by clavulanate. Strain No. 137 produces clavulanate-resistant variant(s) of ESBL(s) too.

P1221 Three New TEM β-lactamases Expressed by Ceftazidime-Resistant *K. pneumoniae* and *E. coli* Isolates from Polish Hospitals

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The objective of the study was to identify extended-spectrum β-lactamases produced by a group of *Enterobacteriaceae* isolates from Poland. 11 *K. pneumoniae* and 1 *E. coli* isolates were collected from 3 hospitals: in Łódź, Rzeszów and Warsaw, at the beginning of 1995. All of them were uniformly resistant to ceftazidime. The RAPD analysis was used to reveal epidemiological relationships among *K. pneumoniae* isolates. Isolates were subjected to ceftazidime-resistance-transfer experiment. β-lactamases expressed by both wild type and transconjugants strains were visualised by IEF. The ceftazidimase activity was assigned to a given IEF band by the bioassay approach. Plasmid DNAs were purified from transconjugants cells and used as templates in PCR reactions with *bla*TEM or *bla*SHV specific primers. Resulting PCR products were sequenced. Ceftazidimases with a pI of 6.0 were detected in extracts of one *K. pneumoniae* and the *E. coli* isolates from Łódź, and 4 *K. pneumoniae* isolates from the clonal outbreak in Rzeszów. These enzymes were expressed by transconjugants. Sequence analysis has revealed 3 different but closely related TEM β-lactamase sequences: TEM-47 (G238S:E240K:T265M), TEM-48 (L21F:G238S:E240K:T265M) and TEM-49 (L21F:G238S:E240K:T265M: G268S). The remainder of 6 *K. pneumoniae* isolates taken to the study were found to produce the SHV-5 ES-β-lactamase. The group of new TEM variants might represent a part of a common branch within the evolutionary tree of TEM β-lactamases.

P1222 ESBL-Producing Strains in a Belgian University Hospital

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Objectives: 1° To evaluate the role of ESBL (extended spectrum β-lactamase) production as mechanism of resistance to third gener-

ation cephalosporins at the AZ-VUB. 2° To determine which detection test can be used routinely. 3° To investigate the genetic relationship between different ESBL positive strains of the same species.

Methods: Three synergism tests, the double disk test (DD), the comparison of disk diffusion zones on MH ± clavulanate (MC) and the Rosco test (RC) combining amoxycillin/clavulanate and cephalosporin disks were applied to 240 selected clinical isolates (132 *Klebsiella* spp., 103 *Enterobacter* spp. and 5 *E. coli*). The reference for an ESBL positive isolate was a MIC ratio ± clavulanate of ≥16 for at least one of the following: cefotaxime, ceftriaxone, ceftazidime, cefepime and aztreonam. ESBL-producers were typed by randomly amplified DNA polymorphism (RAPD).

Results: 107 *Klebsiella* spp. (59 *K. oxytoca* and 48 *K. pneumoniae*), 4 *E. coli*, 2 *E. cloacae* and 1 *E. sakazakii* had at least one MIC ratio ≥16. This corresponds to 15.4% of all *K. pneumoniae* isolates and 31.5% of all *K. oxytoca* (the latter being a mixed population of ESBL-producers and K1-hyperproducers according to their ceftazidime susceptibility). The MC method had the best sensitivity, followed by DD, while RT was not useful. For practical reasons, DD has to be preferred for routine use. The ESBL+ *K. pneumoniae* could be divided in 6 RAPD groups and *K. oxytoca* in 7 but in each species, a predominant group comprised 80% and 60% of strains respectively.

Conclusions: In contrast with other hospitals, in the AZ-VUB, ESBL production is frequent in *K. oxytoca* as well as in *K. pneumoniae* strains. DD seems to be best adapted for routine use. Although several RAPD types were present, the predominance of one type in each species suggests nosocomial transmission.

P1223 Characterization of an Extended-Spectrum β-lactamase Isolated from a Nosocomial *E. coli* Strain

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Objectives: Identification of the mechanisms of resistance to β-lactams of an multiresistant nosocomial *E. coli* strain with a SHV-type behavior.

Methods: Identification and susceptibility testing were performed by API 32GN (BioMérieux) and Kirby-Bauer disk diffusion tests. The MICs were determined by E-tests (AB Biodisk) performed on Mueller-Hinton agar plates (BioMérieux). IEF was performed in precast polyacrylamide gels (pH range, 3 to 9) by using a Pharmacia (Uppsala) PhastSystem apparatus. Nitrocefin (GlaxoWelcome) was used for detection of β-lactamase activity. Recombinant DNA techniques were performed as described by Sambrook et al. (1989). Nucleotide sequence was determined by the dideoxynucleoticle chain termination method by using T7 DNA polymerase (Pharmacia) and bla SHV-5 specific primers.

Results: *E. coli* FF750 with a SHV-type behavior, which is characterized by high resistance to ceftazidime, aztreonam and cefotaxime, produce two β-lactamases of isoelectric point 5.4 and 8.2 coded by different conjugative plasmids. By sequencing the pI 8.2 β-lactamase gene we found the typical mutations of SHV-5 enzyme: 238 glicine by serine and 240 glutamic acid by lisine.

The plasmids encoding SHV-5 and TEM-1 contains sequences encoding the aminoglycosides inactivating enzymes AAC (3)-Va, APH (3′)-Ia and AAC (6′)-Ib

Conclusions: After this first report of an SHV-5 producing *E. coli* strain isolated at a portuguese hospital, epidemiology studies will be required for controlling its spread.

P1224 Survey of Extended Spectrum β-lactamases (ESβL) in *Enterobacteriaceae* Clinical Isolates from North Italian Hospital

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Objectives: ESβLs are widely spread enzymes. The extent to which this happens varies with time, with the hospital or unit type and with the usage of newer cephalosporins. So we have carried out a survey of ESβLs in enterobacteria from our hospital over one year period to evaluate their significance among the pathogen isolates in various wards.

Methods: Many ESβL producing isolates are not resistant to newer cephalosporins or aztreonam in routine susceptibility tests, this is disturbing since ESβLs have been associated with clinical failures even when only low-level resistance was apparent *in vitro*. So we have studied the ESβL production in 620 enterobacteria from patients in ICUs, clinical and surgical wards, apart from the expression of resistance, by GNS-LM card (VITEK System, bioMérieux). The double disk test was also performed. As quality controls we have chosen strains of *E. coli* producing ESβLs group 2be and non-ESβLs.

Results: ESβLs were detected in 15% of isolates with incidence values ranging from 29.5% in *S. marcescens* to 11.2% in *P. mirabilis*. (13.3% *E. coli*, 12.3% *K. pneumoniae*). The data obtained revealed that *S. marcescens* and *Enterobacter* spp. are emerging as ESβL producers at our hospital. Moreover up to 50% of ESβL producing *E. coli* resulted to be susceptible to ceftazidime and aztreonam with pharmacological breakpoints. During the survey was also detected the epidemic spread of TEM-8 like producing *K. pneumoniae* at an ICU.

Conclusions: Our results confirmed the need of a constant monitoring of ESβLs in *Enterobacteriaceae* to prevent and control their dissemination.

P1225 Emergence of an Imipenem Resistant *Klebsiella pneumoniae*

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The North-East of Scotland is currently experiencing an outbreak of multiply-resistant *K. pneumoniae* (MRK), capsular type K_2 with extended spectrum β-lactamase activity, sensitive only to carbapenems, amikacin and colistin. During the outbreak a patient with leukaemia was found to have septicaemia caused by MRK (strain A) and was treated with imipenem for 10 days. Two days after the completion of therapy an imipenem resistant (IMP-R) isolate (strain B) believed be the MRK was recovered from a stool sample. Seventeen days after this an imipenem sensitive variant was recovered from a further stool sample and the IMP-R variant was never isolated from this patient again. Careful epidemiologic investigations have failed to detect any environmental or patient spread of the imipenem resistant organism. The following MIC values (μg/ml) were determined by the NCCLS microdilution method.

Strain	imipenem	meropenem	cefotetan	cefotaxime (ctx)	ctx/clav. acid
A	0.06	0.016	0.25	8	0.125
B	4	8	>32	>1024	1024

MIC values of 0.5 and >32 μg/ml for imipenem were obtained with strains A and B respectively using the E-test method. The isolates were verified as *K. pneumonie* by two methods. The non-motile organisms gave an API 20E profile of 5215773. 16s ribotyping using dye deoxyterminator sequencing also confirmed the identification. Having sequenced 820 nucleotides of the gene, strain B

showed 100% similarity with a randomly selected outbreak MRK and >99% similarity with *K. pneumoniae* NCTC 9633. This is in the first IMP-R organism that we are aware has been confirmed as *K. pneumoniae* by molecular methods. The mechanisms of resistance is being investigated.

P1226 Cefepime and Stepwise Selection of Resistance

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Objectives: comparative selection of resistance to cefepime (FEP), cefpirome (CPO), ceftazidime (CAZ), cefotaxime (CTX) and imipenem (IMP)

Methods: selection was performed on 9 *Pseudomonas aeruginosa* (5 wild phenotype), 5 *Enterobacter cloacae*, 4 *Serratia marcescens* strains by daily transfer in subinhibitory concentrations (subMICs) of antibiotics using a spiral inoculator. Bacterial inoculum was 10^6 cfu/ml and spontaneous mutants were often present with CTX, CAZ in the original population at 4xMIC. They appeared during transfers more rapidly with CPO than with CPM or IMP. Only colonies growing in subMICs were taken for serial passages.

Results: expressed as mean number passages necessary with each selecting antibiotic to reach intermediate (I) or resistance level (R) (number of strains reaching this level out of number tested)

	CTX	CAZ	CPO	FEP	IMP
E. cloacae					
I level	8 (4/5)	10.5 (5/5)	10 (3/5)	28.8 (4/5)	31 (4/5)
R level	11.5 (4/5)	17.2 (4/5)	14 (3/5)	25.5 (2/5)	>30 (0/5)
Ps. aeruginosa (wild phenotype strains)					
I level	3 (4/4)	7 (5/5)	6 (4/4)	11.8 (5/5)	15.8 (5/5)
R level	3.5 (4/4)	18 (4/5)	15 (4/4)	30 (4/5)	27.3 (3/5)
S. marcescens					
I level	13 (3/3)	9.7 (3/4)	18 (3/4)	14.7 (3/4)	30 (1/4)
R level	16.3 (3/3)	20.5 (2/4)	31 (2/3)	19 (2/4)	31 (1/4)

Conclusions: Rate of selection was lower with IMP and FEP. Mutants selected with CTX, CAZ remained susceptible to IMP, FEP and CPO indeed those selected with FEP and CPO were more often cross R to all cephalosporins probably due to higher cephalosporinase production related to slower development of resistance.

P1227 Varied Potentials of β-Lactam Antibiotics in the Selection of Resistance in Clinical *Enterobacter cloacae* Isolates

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Objectives: To test the ability of eight commonly-used and newer β-lactams to select resistant *Enterobacter cloacae* isolates in the search for those with a high antimicrobial activity and a low resistance induction potential for antimicrobial chemotherapy.

Methods: Nine standard and clinical *E cloacae* isolates were subcultured daily in test antibiotics at doubling concentrations from 0.125 × MIC to 12 × MIC. Resistance development was monitored by a disk diffusion test throughout the passages.

Results: All isolates managed to grow in the presence of 12 × MIC of all cephalosporins tested and meropenem while imipenem eradicated all at 8 × MIC. Ceftazidime, ceftriaxone and cephamandole selected resistance at a faster rate than cefoperazone while cefepime, cefpirome, imipenem and meropenem showed relatively low resistance-selection potential. Although cross-resistance was a common phenomenon amongst the resistant mutants, cross-resistance to cefuroxime and cephamandole developed most readily upon selection by other antibiotics but rarely to the carbapenems. The resistance phenotypes of most selected strains remained stable upon serial passages in antibiotic-free medium for 10 days.

Conclusions: Second and third generation cephalosporins selected resistance much faster than fourth generation cephalosporins and carbapenems. Resistant mutants exhibited cross-resistance to cephalosporins but not to carbapenems.

P1228 Role of the Outer Membrane Proteins in the Permeability of Beta-Lactams

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Objectives: To evaluate the role of the outer membrane proteins (OMP) in the resistance to different beta-lactams and beta-lactam plus inhibitor combinations in *Klebsiella pneumoniae* isolates.

Methods: Two *Klebsiella pneumoniae* strains were investigated, one of them produced an SHV-3 extended-spectrum beta-lactamase while the other was non ESBL producer. Strains were identified by ATB (BioMérieux). MICs of antimicrobial agents were determined by microbroth dilution method. Cefoxitin-resistant mutants could easily be selected on cefoxitin containing medium and outer membrane proteins of the mutans were prepared and analysed on SDS-PAGE. Conjugation assay was carried out with *E. coli* J53-2. Beta-lactamase activity was measured by using nitrocefin.

Results: Correlation has been found between the loss of 35-kDa and 36-kDa proteins and decreased susceptibility to beta-lactam and beta-lactamase inhibitor combinations, and resistance to 7-α-methoxy-beta-lactams, aztreonam and extended-spectrum cephalosporins in the SHV-3 producing strain. The OMP alteration alone in strain 53,283 proved to be insufficient to cause resistance to extended-spectrum cephalosporins and beta-lactam plus inhibitor combinations, with the exception of cefoxitin and latamoxef. Mutants obtained from 46,655, showed elevated level of MICs to quinolons.

P1229 ESBL Producing *Escherichia coli* in Neonatal ICU Patients

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Objectives: To determine if *Escherichia coli (E. coli)* strains isolated from clinical specimens which were resistant to ceftazidime in disc diffusion test, were ESBLs producers.

Methods: Strains isolated from urines of 8 patients and blood cultures of 2 patients in neonatal intensive care unit (NICU), were identified as *E. coli* with BBL Crystal E/NF ID Becton Dickinson and RAPID ID 32 E BioMerieux; disc diffusion test was peformed with Kirby-Bauer procedure for amoxicillin, amoxicilin-clavulanic acid, cefotaxime, cefoperazone, ceftriaxone, ceftazidime, gentamicin and amikacin; than MICs were determined with agar dilution technique for amoxicillin, amoxicilin-clavulanic acid, cefotaxime, cefoperazone, ceftriaxone, ceftazidime, and "double-disc diffusion test" with amoxicillin-clavulanic acid (20/10 μg) and ceftazidime (30 μg) discs. QC strain was *E. coli* ATCC 25,922.

Results: MICs of all strains for amoxicilin were >1024 μg/ml, for cefotaxime, cefoperozone, ceftriaxone and ceftazidime from 32 to >1024 μg/ml, and for amoxicillin-clavulanic acid from <1 to 256 μg/ml. Double disc diffusion test showed in all the strains synergistic effect of ceftazidime and clavulanic acid. All strains were resistant to aminoglycosides.

Conclusion: ESBL-producing aminoglycosides resistant *E. coli* strains isolated from clinical specimens in our NICU are first such strains proven in Croatia and should be of great concern in NICU patients.

Candida infections

P1230 Candidaemia: A Cause or Effect of Staph. Septicaemia!

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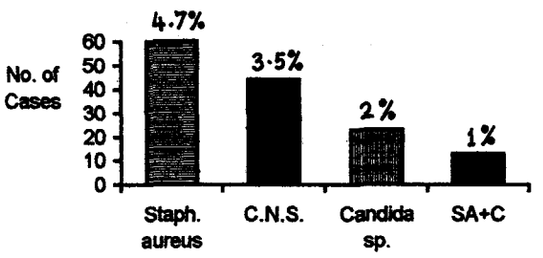
Objectives: *Staph. aureus* and *Candida* septicaemia cases were pursued to evaluate their causal relationship to each other.

Methods: 1255 blood cultures were studied, using BacT/Alert (Organon-Teknika) Blood Culture system. Isolation of *Staph. aureus* (SA), *Coagulase Negative Staph.* (CNS), *Candida sp.* (C) and mixture of organisms SA+C or C+SA were evaluated as per the above objective.

Results:



CNS cases had or developed Candidaemia. 10/13, SA+C cases started with *Staph. aureus* (6) or *Candida* (4) septicaemia and progressed to SA+C or C+SA on follow up. 2/36 *Candida* cases, had mixed septicaemia other than *Staph. aureus*, like *E. coli, Enterobacter sp.*, etc. whereas 13/36 cases developed SA+C septicaemia.

Conclusions: The above suggests a cause and effect relationship of *Staph. aureus* and *Candida sp.* infections probably by way of producing mutually beneficial chemotactic or growth factors that need further study.

P1231 Candida Sepsis in Patients with HIV Infection

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Objective: to evaluate the risk and prognostic factors of the sepsis due to different species (spp.) of *Candida* in HIV infected patients admitted at our ward from 1989 to 1996.

Methods: a case control study was performed. A control group of 64 patients with AIDS and without sepsis was included in the study.

Results: we observed 32 HIV infected patients (the large majority of them were males, intravenous drug abusers and with AIDS) with Candida spp. sepsis. The isolated strains were *C. albicans* in 18 episodes. (eps., 56%), *C. tropicalis* in 4 eps. (13%), *C. glabrata* in 3 eps. (9%), *C. parapsilosis, C. krusei* and *C. guilliermondii* in 2 eps (6%), respectively and *C. zeylanoides* in 1 eps. Univariate analysis of predisposing factors indicated that the low CD4+ T cell level <100/mmc ($P = 0.02$), the CVC usage ($P = 0.02$) and the neutropenia (neutrophils count <1000/mmc) ($P = 0.04$) were risk factors associated with the development of *Candida* sepsis. The response to therapy was favourable in 22 eps. (69%); death occurred in 10 eps. (31%), while a recurrence occurred in only 1 eps. The outcome of sepsis was influenced by the low number of CD4+ T cell ($P = 0.01$) and of neutrophils ($P = 0.03$).

Conclusions: our data suggest that *Candida* sepsis is an important event in HIV-infected patients, especially under particular conditions (e.g. CVC usage, neutropenia and low CD4+ T cell level) and it requires a special attention from the physicians who must early recognise and treat this condition.

P1232 Candidemia: A Retrospective Study

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Objective: To evaluate, retrospectively, bloodstream episodes of *Candida* spp. along one year (1.995).

Methods: Hemocultures were incubated in Bact Alert System. Positive samples were subcultured in Saboureaud agar (Biomerieux) and yeast isolates were identified by Vitek System (Biomerieux). *C. parapsilopsis* antifungal susceptibilities were assayed by E-test.

Results: Total number of candidemias along 1.995 was twenty-five belonging to twentythree hospitalized patients. The yeast more oftenly isolated was *C. parapsilopsis* (52%) followed by *C. albicans* (32%). Other *Candida* species found were *C. tropicalis, C. glabrata* and *C. lusitaniae.* Fungemia by *C. parapsilopsis* was significatively associated to tumoral and pediatric patients (36% and 44%, respectively). We have not found any association between candidemia and age of patients.

All *C. parapsilopsis* strains were sensitive to ketoconazole, fluconazole, itraconazole, amphotericine B and flucitosine (MIC 90 1 mcg/mL).

Conclusions:

(1) *C. parapsilopsis* has just emerged as an important nosocomial pathogen in our hospital.
(2) The prevalent yeast isolated from fungal blood infections is *C. parapsilopsis.*
(3) *C. parapsilopsis* strains isolated from hospitalized patients are sensitive to the main systemic antifungal drugs.

P1233 *Candida spp.* an Important Pathogens in Nosocomial Infections

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Objectives: The aim of this study was to determine the influence of intensified antimicrobial therapy and intensive care measures on the incidence of *Candida spp.* isolated from different clinical specimens.

Methods: We analysed 77 872 records of our clinical microbiology laboratory from 1.1.1994 to 31.12.1995.

Results: The incidence of *Candida spp.* in clinical materials was higher about 36% in 1995 than 1994. *Candida spp.* was the third most common organism recovered from the blood cultures in 1995, much increase comparing 1994 (the seventh place). *Candida albicans* is still the predominant pathogen among the genus *Candida* in our hospital. Two hospital units: ICU and Haematological Department had the most significant increase in the incidence of *Candida spp.* Interestingly, in 1995 we observed a high increase in the incidence of *Candida (Torulopsis) glabrata* recovered from different clinical specimens. *Candida (Torulopsis) glabrata* represented 9% of all yeast isolates therefore special attention should be paid to the treatment of fungal infections in our hospital (this species is frequently resistant to fluconazol). This increase is probably associated with a broad use of fluconazol in prophylaxis and empirical treatment of fungal infections in our hospital.